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ORIGINAL RESEARCH

Epidemiological Characteristics and Carbapenemase Analysis of Carbapenem-Resistant Enterobacterales Isolates in a Teaching Hospital in Guangzhou, China

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Background: In this study, carbapenem-resistant Enterobacterales (CRE) were isolated from a teaching hospital in Guangzhou between January 2020 and March 2023, meticulously examining the antimicrobial resistance patterns, carbapenemase types, and epidemiological characteristics of these isolated strains. This comprehensive analysis serves as an invaluable insight for optimizing CRE treatment strategies for clinical practitioners and implementing robust measures to prevent and control nosocomial infections within healthcare settings.

Methods: The antimicrobial susceptibility testing aimed to ascertain carbapenem resistance in Enterobacterales, while the production of carbapenemase was assessed through rapid phenotypic identification by immunochromatographic assay (KPC, NDM, VIM, IMP, and OXA-48-like) and confirmed by PCR.

Results: Among the 300 CRE strains collected from January 2020 to March 2023, *Klebsiella pneumoniae* (CR-Kpn) accounted for 72.7%, *Escherichia coli* (CR-Eco) 12.3%, *Enterobacter cloacae* (CR-Ecl) 8.3%, *Klebsiella aerogenes* (CR-Eae) 2.3%, *Citrobacter freundii* (CR-Cfr) 2.0%, and others 2.3%. Among the five carbapenemase types, *bla*KPC-like ranked first accounting for 66.7%, followed by *bla*NDM-like (23.0%), *bla*OXA-48-like (0.7%), and *bla*IMP-like (0.7%), of which six strains of *bla*KPC-like plus *bla*NDM-like were detected simultaneously. Although *bla*KPC-like predominated in adults and the elderly, *bla*NDM-like was more common in children. These CRE strains showed high resistance to most antibiotics; however, they showed high sensitivity to tigecycline and colistin.

Conclusion: CRE strains exhibited a high resistance rate of multiple antibacterial drugs, and *bla*KPC-like were widely prevalent in CRE strains, particularly *K. pneumoniae*. Clinical attention should be paid to the rational use of antibacterial drugs, and CRE monitoring and hospital infection prevention and control should be continuously strengthened.

Keywords: CRE, KPC, antimicrobial resistance

Introduction

Carbapenem-resistant Enterobacterales (CRE) poses an escalating threat to human health, giving more and more attention around the world.[1,2] At present, as CRE shows high resistance to most antimicrobials, the life safety of patients is seriously threatened when they were infected with CRE.[3–5] The United States Centers for Disease Control and Prevention (CDC) defines CRE as Enterobacterales that exhibit in vitro resistance to any carbapenems.[6]

In general, CRE may be divided into carbapenemase-producing CRE (CP-CRE) and non-carbapenemase-producing CRE (non-CP-CRE), with CP-CRE receiving the most attention.[7] CPE has been widely disseminated in many regions around the world. In particular, the infection rate is high in the Mediterranean, South and Southeast Asia, South America, etc.[8,9] In CP-CRE strains, the carbapenemase gene is often carried on a mobile genetic element, significantly increasing the likelihood of horizontal gene transfer. Moreover, plasmids in CP-CRE frequently harbor additional resistance determinants potentially leading to

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multidrug resistance across various drug classes.[10–12] In non-CP-CRE, increased expression of genes encoded by extended-spectrum β-lactamases (ESBL) or AmpC enzymes in combination with outer membrane porin impermeability and active efflux mechanisms, which were closely related to the rapid development of non-CP-CRE.[13–15]

In CRE, carbapenem-resistant *Klebsiella pneumoniae* and *Escherichia coli* are commonly encountered, followed by *Enterobacter cloacae* in China.[14,16–18] Carbapenemases are generally divided into three types, including class A, B, and D. The Ambler class A β-lactamase *Klebsiella pneumoniae* carbapenemase (KPC) is predominantly epidemic in the United States, Colombia, Ecuador, Greece, and Portugal in Europe,[19–21] and KPC-2 is the most prevalent in carbapenem-resistant *K. pneumoniae* isolates in China.[22] The class B carbapenemases, also known as metallo-β-lactamases (MBLs), including the New Delhi metallo-β-lactamases (NDM), IMP (active on imipenem)-type carbapenemases, and Verona integron-encoded MBLs (VIM), are primarily found in the Middle East and South Asia.[19,23,24] OXA-48-like-producers are the common variant of class D carbapenemases, which are prevalent in the Middle East, Europe, and North Africa.[19,25] These genes of carbapenemases are easily transmitted through plasmids and transposons.[26]

In this study, the CRE strains we collected were mainly predominantly *K. pneumoniae* from 2020 to 2023 and *bla*KPC-like was widely prevalent. The study delved into the antimicrobial resistance profiles, types of carbapenemases, and the epidemiological characteristics of these isolated strains. Eventually, our study will offer more invaluable insights that are pivotal for prevention, control, and clinical management of CRE infections.

Materials and Methods

Bacterial Isolates

A total of 300 consecutive CRE strains were collected from patients at the First Affiliated Hospital of Guangzhou Medical University, a teaching hospital in Southern China, from January 2020 to March 2023. The duplicate isolates obtained from different parts or inpatient units of same patient were excluded. These strains exhibited resistance to at least one carbapenem (including meropenem, imipenem, and ertapenem) and underwent isolation, purification, and storage at −80 °C freezers. In addition, clinical information pertaining to these patients was obtained through the hospital's electronic medical record system.

Identification and Antimicrobial Susceptibility Testing

All collected isolated strains were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (bioMerieux, Inc., France). The antimicrobial susceptibility testing was performed using automatic microbial identification and susceptibility system VITEK 2XL compact (bioMerieux, Inc., France) and the Kirby–Bauer method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guideline.[27] The results of antibiotic susceptibility assays were interpreted in accordance with CLSI M100. In addition, the breakpoint of tigecycline for Enterobacteriaceae and colistin was based on the FDA and USCAST standards,[28] respectively. Pseudomonas aeruginosa ATCC 27853 and E. coli ATCC 25922 served as quality control standards for antimicrobial susceptibility testing.

Immunochromatography Assay and PCR Testing for Detection of Main Carbapenemases

All CRE isolates were subjected to carbapenemase (KPC, NDM, VIM, IMP, and OXA-48-like) detection using the immunochromatography assay (name of the kit, Beijing Gold Mountain River Tech Development Co., China) following manufacturer's instructions and using home made PCR. The primer sequences used for PCR of the five carbapenemases were as follows:[29] *bla*KPC-like forward primer 5′-TGT CAC TGT ATC GCC GTC-3′ and reverse primer 5′-CTC AGT GCT CTA CAG AAA ACC-3′; *bla*NDM-like forward primer 5′-GCA GCT TGT CGG CCA TGC GGG C-3′ and reverse primer 5′-GGT CGC GAA GCT GAG CAC CGC AT-3′; *bla*OXA-48-like forward primer 5′-GCG TGG TTA AGG ATG AAC AC-3′ and reverse primer 5′-CAT CAA GTT CAA CCC AAC CG-3′; *bla*VIM-like forward primer 5′-GTT TGG TCG CAT ATC GCA AC-3′ and reverse primer 5′-AAT GCG CAG CAC CAG GAT AG-3′; *bla*IMP-like forward primer 5′-GAA GGC GTT TAT GTT CAT AC-3′ and reverse primer 5′-GTA CGT TTC AAG AGT GAT GC-3′.

Statistical Analysis

Descriptive statistics were used to summarize the epidemiologic characteristics of CRE strains. For categorical variables, the percentage of CRE strains in each category was calculated. All analyses were performed using WHONET (version 5.6) and GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA).

Results

Distribution and Clinical Features of CRE Strains

Of the 300 strains of CRE, the majority were isolated from elderly patients, with a significant proportion being male (76.0%). These strains are predominantly originated from intensive care unit (33.0%), respiratory medicine (25.6%), and urology surgery (8.3%, Table 1). In the bacterial distribution, *K. pneumoniae* ranked firstly with 218 strains (72.7%), followed by *E. coli* (37, 12.3%), *E. cloacae* (25, 8.3%), seven strains of *K. aerogenes* (2.3%), *Citrobacter freundii* (6, 2.0%), and other Enterobacterales (7, 2.3%; Figure 1A). The predominant sources of these bacterial specimens were sputum samples (60.7%), particularly those obtained through bronchoscopy, which constituted the highest proportion at 23.7%, followed by urine (14.0%) and blood (7.7%, Figure 1B). Among them, *K. pneumoniae* was primarily isolated from sputum (69.3%), whereas *E. coli* and *E. cloacae* were mainly derived from urine (29.7% and 32.0%, Figure 1C). In this study, invasive infections accounted for approximately 19.7% of all CRE-related infections, including bacteremia and intra-abdominal infection, with *K. pneumoniae* being the primary pathogen responsible for infection accounting for 67.8% of invasive infections and 73.9% of non-invasive infections (Figure 1D).

Antimicrobial Susceptibility

The antimicrobial susceptibility results are presented in Table 2. The CRE strains showed a high sensitivity to colistin (96.2%), followed by tigecycline (82.0%), amikacin (47.3%), and sulfamethoxazole (30.7%). However, these strains exhibited significant resistance to most third- and fourth-generation cephalosporins, β-lactamase combinations such as piperacillin–tazobactam and cefoperazone–sulbactam, as well as carbapenems. Moreover, notable variations were observed among different species, for example, the sensitivity rate of *K. pneumoniae* to amikacin is merely 32.1% compared with *E. coli* (73.0%) and *E. cloacae* (100%). Notably, compared to *K. pneumoniae* and *E. cloacae, E. coli* exhibited higher sensitivity to tigecycline (97.3%). In addition, *K. pneumoniae* showed greater resistance to aztreonam

Table 1 Clinical Distribution of CRE Strains

Category	Distribution	Number(n)	Proportion(%)
Age	0~14	15	5.0
	15~60	100	33.3
	More than 60	185	61.7
Gender	Male	228	76.0
	Female	72	24.0
Ward	Intensive care unit	99	33.0
	Respiratory medicine	77	25.7
	Urology surgery	25	8.3
	Hepatobiliary surgery	15	5.0
	Internal medicine	12	4.0
	Pediatrics	11	3.7
	Thoracic surgery	6	2.0
	Others*	55	18.3

Notes: *Including 5 strains are from Allergy, Emergency and Cardiac surgery, respectively; 4 strains each from the Department of Cardiology, the Department of Organ Transplantation, the Department of Geriatrics, the Department of Nephrology, and the Department of Hematology; 3 strains each from Neurology, Neurosurgery, Gastrointestinal surgery and General surgery; 2 strains each in the Department of General Medicine, Vascular Surgery, Plastic Surgery, and Specialist Outpatient Clinic.

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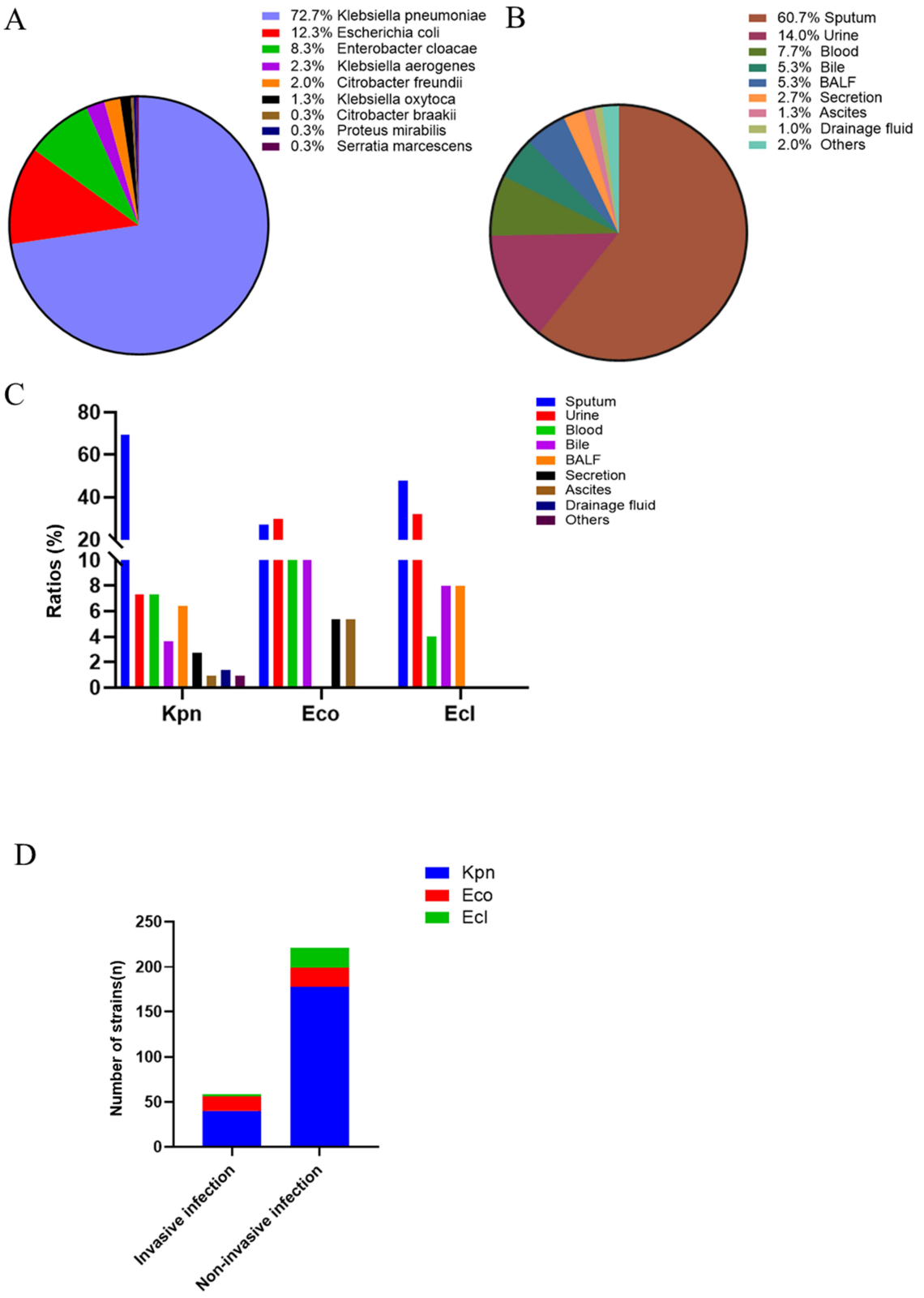

Figure 1 (**A**) Distribution ratios of CRE strains. (**B**) Proportion of specimens from CRE strains. (**C**) Proportion of specimens for the main strains of *Klebsiella pneumoniae, Escherichia coli* and *Enterobacter cloacae*. (**D**) Quantities of *Klebsiella pneumoniae, Escherichia coli* and *Enterobacter cloacae* strains between invasive and non-invasive infections.

Table 2 Antimicrobial Susceptibility

Antibiotic	All strains (n=300)		Kpn (n=218)		Eco (n=37)		Ecl (n=25)	
	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)
Amoxicillin-Clavulanic Acid	99.3	0.4	99.1	0.5	100.0	0.0	100.0	0.0
Amikacin	51.0	47.3	66.5	32.1	21.6	73	0.0	100
Ampicillin	100.0	0.0	100.0	0.0	100	0.0	100.0	0.0
Aztreonam	87.5	12.2	95.1	4.9	70.6	29.4	47.1	47.1
Ceftazidime	97.7	1.7	97.2	2.3	97.3	0.0	100	0.0
Ciprofloxacin	95.6	4.1	97.6	2.4	97.1	2.9	88.2	5.9
Colistin	3.0	96.2	3.5	96.0	0.0	100	0.0	100.0
Ceftriaxone	98.9	0.7	98.6	0.9	100.0	0.0	100.0	0.0
Cefoperazone-Sulbactam	95.0	3.3	95.9	1.8	94.6	5.4	96.0	4.0
Cefuroxime	99.3	0.7	99.1	0.9	100.0	0.0	100.0	0.0
Cefazolin	100	0.0	99.1	0.9	100.0	0.0	100.0	0.0
Doxycycline	70.5	17.0	69.8	17.1	97.1	0.0	47.1	29.4
Ertapenem	97.0	0.7	98.2	0.5	94.4	2.8	96.0	0.0
Cefepime	92.0	4.7	96.3	3.2	89.2	0.0	62.5	16.7
Cefoxitin	96.5	2.1	96.3	1.8	94.4	5.6	100	0.0
Gentamicin	65.2	32.8	76.5	22.3	54.8	38.7	17.6	82.4
Imipenem	87.7	10.0	92.2	6.0	78.4	16.2	60.0	36.0
Levofloxacin	89.9	6.4	95.8	1.4	89.2	5.4	64.0	32.0
Meropenem	90.3	8.1	94.9	3.2	81.1	16.2	64.0	36.0
Minocycline	78.2	10.7	80.0	9.3	88.2	0.0	64.7	29.4
Trimethoprim-Sulfamethoxazole	69.3	30.7	71.1	28.9	78.4	21.6	52.0	48.0
Ticarcillin-Clavulanic Acid	98.2	0.4	99.0	0.0	91.2	2.9	100.0	0.0
Tigecycline	5.8	82.0	5.6	80.3	2.7	97.3	4.2	75.0
Tobramycin	67.9	23.2	77.1	19.5	47.1	23.5	29.4	41.2
Piperacillin-Tazobactam	98.7	1.0	99.5	0.5	97.3	2.7	95.8	0.0

(95.1%) than *E. coli* (70.6%) and *E. cloacae* (47.1%). Furthermore, the resistance rate of *E. coli* toward doxycycline was remarkably high at 97.1%, surpassing that of *K. pneumoniae* and *E. cloacae*, which did not exceed 70%.

Carbapenemase Characteristics of CP-CRE Strains

Among the collected strains, the proportions of CP-CRE and non-CP-CRE were 93.3% and 6.7%, respectively. According to PCR testing, *bla*KPC-like was the predominant carbapenemase (66.7%), followed by *bla*NDM-like (23.3%), *bla*KPC plus NDM-like (2.0%), *bla*IMP-like (0.7%), and *bla*OXA-48-like (0.7%). Secondly, in *K. pneumoniae* strains, *bla*KPC-like accounted for 84.4%, whereas *bla*NDM-like, *bla*KPC-like plus *bla*NDM-like, and *bla*OXA-48-like accounted for 6.9%, 2.3%, and 0.9% respectively. *bla*NDM-like, *bla*KPC-like, and the combination of *bla*KPC-like plus *bla*NDM-like accounted for 75.7%, 8.1%, and 2.7% of *E. coli* strains, respectively. In *E. cloacae,* *bla*NDM-like and *bla*KPC-like were detected at 60.0% and 28.0%, respectively (Figure 2A–D). In addition, one IMP-producing strain was identified in *Klebsiella oxytoca* and *Serratia marcescens*. Subsequently, rapid immunochromatography was performed, showing sensitivity and specificity, of 98.5% and 94.3%, respectively (Table 3).

Carbapenem MIC Values of Different Carbapenemases of CRE Strains

The MIC values for carbapenems varied according to carbapenemase types. As shown in Figure 3A and B, *bla*KPC-like and *bla*NDM-like of the strains demonstrated higher resistance levels compared with *bla*IMP-like and *bla*OXA-48-like against meropenem and imipenem. Furthermore, 94.0% of *bla*KPC-like strains and 92.1% of *bla*NDM-like strains exhibited high levels of resistance to meropenem (MIC≥16 μg/mL), whereas only 1.6% of *bla*KPC-like strains and 6.3% of *bla*NDM-like strains showed moderate levels of resistance (MIC=8 μg/mL) to meropenem. Similarly, 91.7% of *bla*KPC-like strains and

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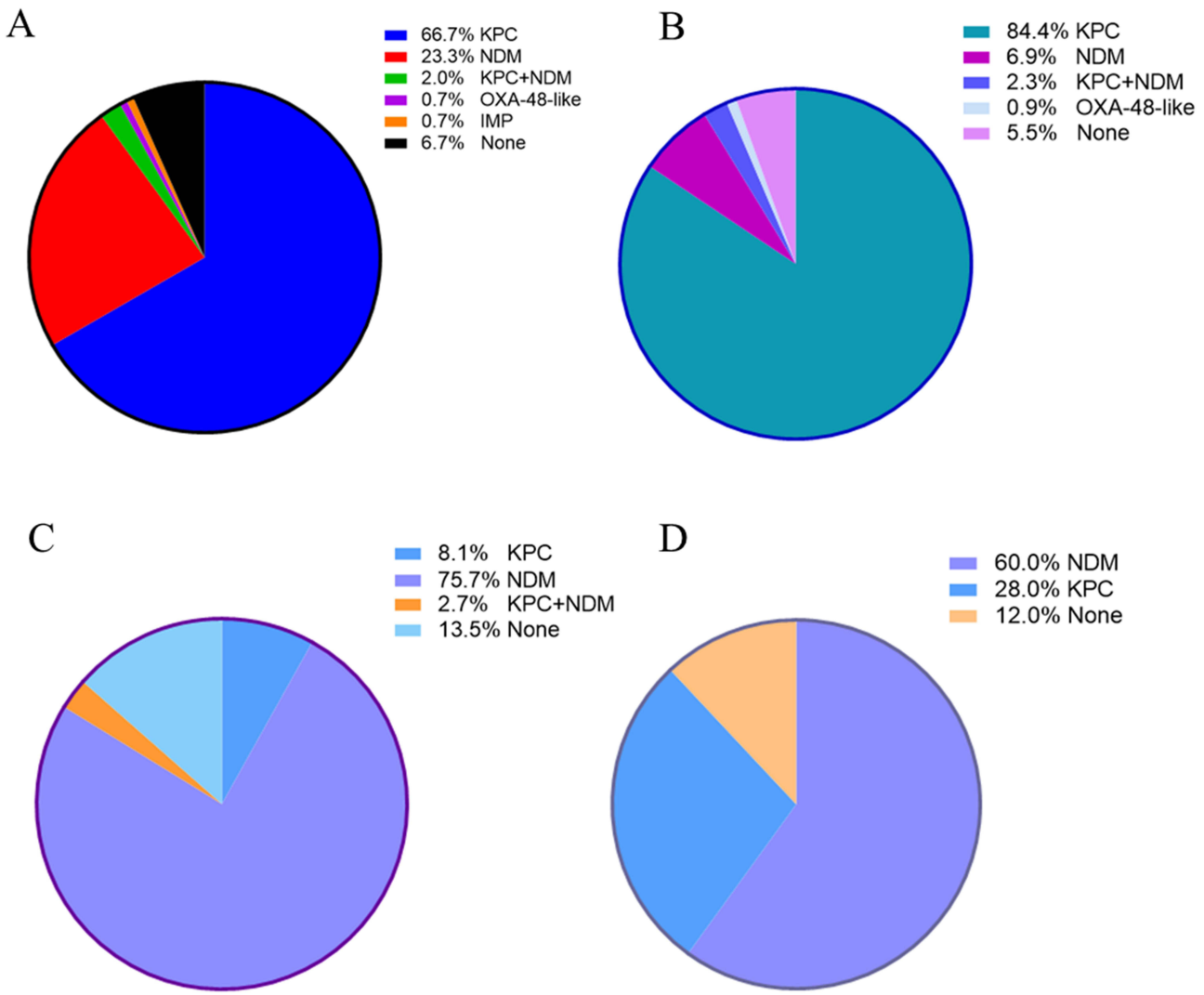

Figure 2 Ratios of various carbapenemase of CRE strains (**A**), *Klebsiella pneumoniae* strains (**B**), *Escherichia coli* strains (**C**), *Enterobacter cloacae* strains (**D**).

89.6% of *bla*NDM-like strains displayed high levels of resistance to imipenem (MIC≥16 μg/mL), with only 3.6% of *bla*KPC-like strains and 7.5% of *bla*NDM-like strains showing moderate levels of resistance (MIC=8 μg/mL). In addition, the resistance patterns among different carbapenemases were analyzed within the same strain toward carbapenems, revealing that *K. pneumoniae* exhibited a higher degree of resistance to meropenem when associated with *bla*KPC-like rather than *bla*NDM-like (Table 4).

Table 3 Evaluation of the Diagnostic Performance of Rapid Immunochromatography for Carbapenemases

Carbapenemase	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
KPC	98.5	94.3	97.0	97.1
NDM	100.0	100.0	100.0	100.0
OXA-48-like	100.0	100.0	100.0	100.0
IMP	100.0	100.0	100.0	100.0
VIM	/	/	/	/

Abbreviations: PPV, positive predict value; NPV, negative predict value; /, Not detected.

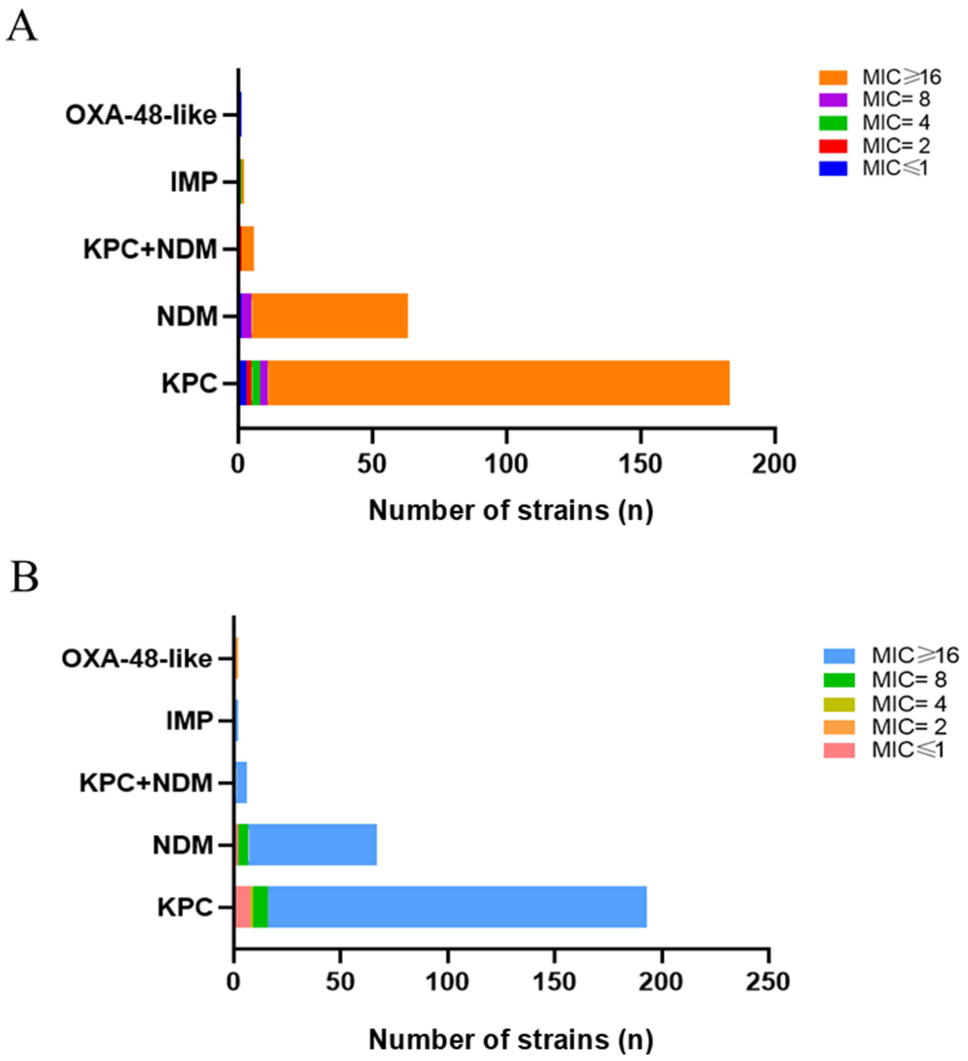

Figure 3 MIC values of CRE strains against Meropenem (**A**) and Imipenem (**B**).

Clinical Features According to Carbapenemase Type

In children, *bla*NDM-like was the most prevalent carbapenemase, with *bla*NDM-like-producing *K. pneumoniae* (NDM-Kpn) accounting for 26.7% and *bla*NDM-like -producing *E. coli* (NDM-Eco) accounted for 20.0%. On the contrary, *bla*KPC-like -producing *K. pneumoniae* (KPC-Kpn) was common among young and old people, accounting for 47.0% and 73.5%, respectively (Figure 4A and B). Among the invasive infections, *bla*KPC-like-producing *K. pneumoniae*

Table 4 Sensitivity of Different Carbapenemases of Kpn, Eco and Ecl Strains to Carbapenems

Carbapenemase	Meropenem					Imipenem				
	S	I	R	MIC=8	MIC≥16	S	I	R	MIC=8	MIC≥16
KPC-Kpn	0.0	1.1	98.9	1.1	97.2	1.6	0.0	98.4	3.9	95.1
NDM-Kpn	0.0	0.0	100.0	23.1	76.9	6.7	0.0	93.3	6.7	86.7
KPC+NDM-Kpn	0.0	20.0	80.0	0.0	80.0	0.0	0.0	100.0	0.0	80.0
OXA-48-like-Kpn	50.0	0.0	50.0	/	/	0.0	100.0	0.0	/	/
KPC-Eco	66.7	0.0	33.3	0.0	0.0	66.7	0.0	33.3	0.0	50.0
NDM-Eco	3.6	3.6	92.9	3.7	92.6	3.6	3.6	92.9	11.1	85.2
KPC-Ecl	85.7	0.0	14.3	50.0	0.0	85.7	14.3	0.0	0.0	0.0
NDM-Ecl	0.0	0.0	100.0	0.0	100.0	0.0	0.0	100.0	7.1	92.9

Abbreviation: /, Not detected.

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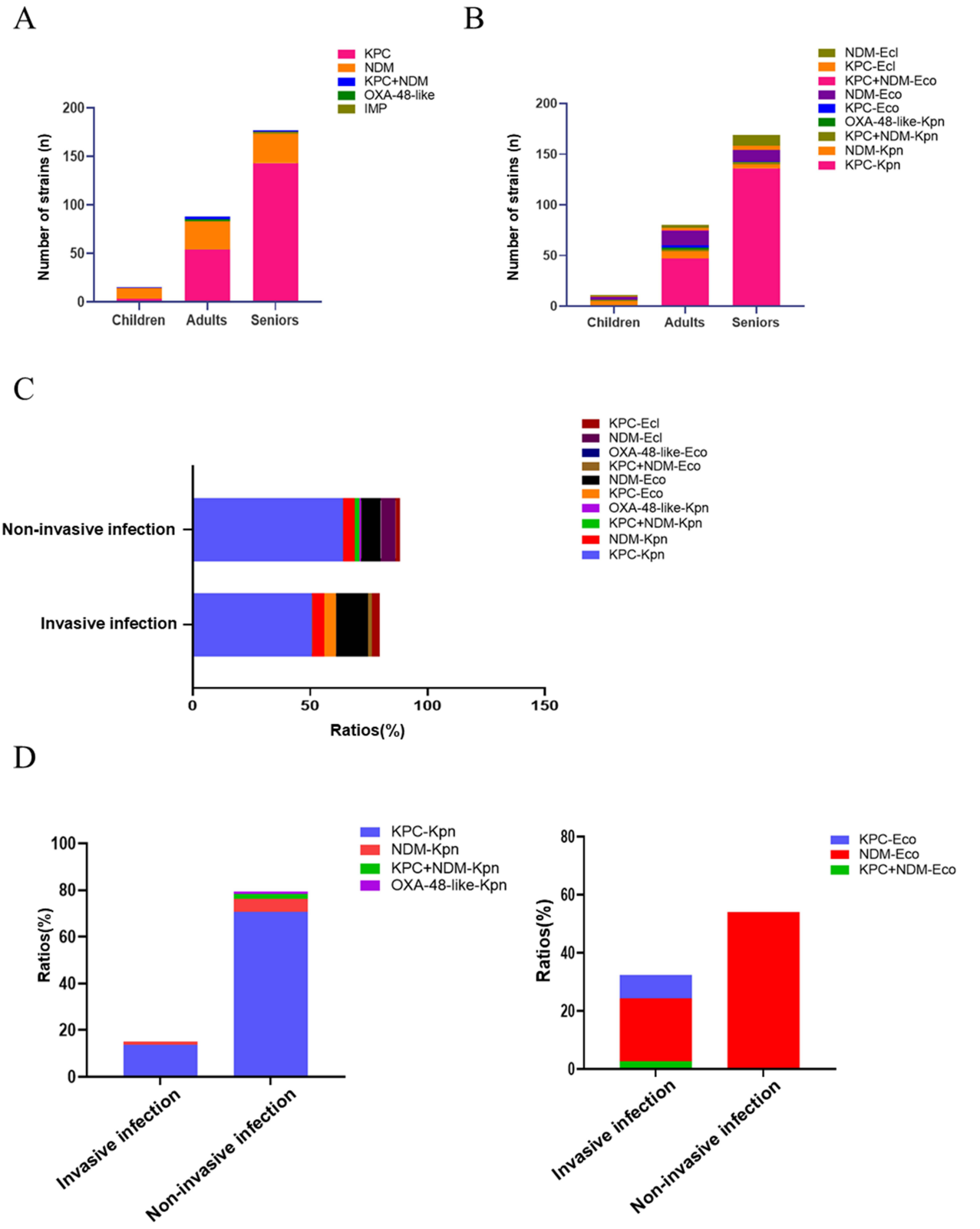

Figure 4 (**A**) Distribution of carbapenemase in different age groups. (**B**) The distribution of different carbapenemase in Kpn, Eco, Ecl strains at different ages. (**C**) The ratios of carbapenemase of Kpn, Eco, Ecl strains between invasive and non-invasive infection. Proportion of carbapenemase in Kpn (**C**) and Eco (**D**) strains in invasive versus non-invasive infections.

accounted for 50.8%, followed by *bla*NDM-like-producing *K. pneumoniae* (5.1%), *bla*KPC-like-producing *E. coli* (KPC-Eco, 5.1%), *bla*NDM-like-producing *E. coli* (13.6%), *bla*KPC-like+ *bla*NDM-like-producing *E. coli* (KPC+NDM-Eco, 1.7%), and *bla*KPC-like-producing *E. cloacae* (KPC-Ecl, 3.4%). In addition, 68.5% of invasive infections were dominated by *bla*KPC-like strains, including *bla*KPC-like-producing *K. pneumoniae* (63.9%) and *bla*KPC-like-producing *E. cloacae* (2.1%), followed by *bla*NDM-like-producing *E. coli* (8.3%), *bla*NDM-like-producing *E. cloacae* (NDM-Ecl, 6.2%), and *bla*NDM-like-producing *K. pneumoniae* (5.0%). *bla*KPC-like+ *bla*NDM-like-producing *K. pneumoniae* (KPC+NDM-Kpn) and *bla*OXA-48-like-producing *K. pneumoniae* (OXA-48-like-Kpn) accounted for 2.1% and 0.8% of the non-invasive infections, respectively. Moreover, *K. pneumoniae* remained the primary pathogen in CRE, accounting for 67.8% of invasive infections and 73.9% of non-invasive infections, respectively. Therefore, among *K. pneumoniae*-associated infections, *bla*KPC-like-producing *K. pneumoniae* (70.1%) predominated in non-invasive infections, whereas it accounted for only 13.8% of invasive infections. However, the prevalence of invasive infection caused by *bla*NDM-like-producing *E. coli* reached 21.6% among *E. coli*-associated infections (Figure 4C and D).

Discussion

Carbapenem-resistant Enterobacterales have emerged as a significant global concern, with the spread of CRE posing a major threat to global health.[30–32] Furthermore, CRE infection is frequently associated with poor prognosis, limited treatment options, and increased mortality rates.[31,33,34] Therefore, comprehensively understanding carbapenemases of CRE, epidemic trends, and infection characteristics were urgently needed. In this article, CRE strains are mainly composed of *bla*KPC-like-producing *K. pneumoniae, bla*NDM-like-producing *E. coli*, and *bla*NDM-like-producing *E. cloacae*. Notably, *bla*KPC-like-producing *K. pneumoniae* remains the most prevalent pathogen in invasive and non-invasive infections, thereby necessitating heightened attention toward it.

In CRE strains, carbapenemase-producing CRE is the most common compared with non-carbapenemase-producing CRE, which may be due to the fact that their carbapenemase-producing genes can be transmitted through mobile elements or transposons.[10,35,36] In addition, the proportion of CPE in CRE has exceeded 60%,[37,38] whereas in this study, 93.3% of CPE strains were present, indicating that carbapenemase production remains the dominant mechanism in this region. Furthermore, KPC emerged as the predominant carbapenemase in adults, accounting for 66.7% of the total. Among various bacterial strains, *bla*KPC-like-producing *K. pneumoniae* accounted for a substantial majority at 84.4%, whereas *bla*KPC-like-producing *E. coli* and *bla*KPC-like-producing *E. cloacae* accounted for 8.1% and 28.0%, respectively.

Based on the literature, KPC is the predominant carbapenemase among adult patients.[22,39] In this study, a prevalence of 27.0% and 71.5% for *bla*KPC-like was observed in young and elderly individuals, respectively. Moreover, our findings indicated that *bla*KPC-like-producing *K. pneumoniae* remained the most common among adults (47.0%–73.5%), which is consistent with previous literature reports.[40] However, *bla*NDM-like-producing *K. pneumoniae* (26.7%) was more frequently detected in children, indicating differences in carbapenemase types between these age groups. Following the discovery of the first *bla*KPC-like-producing *E. cloacae* strain in Shanghai in 2010, various carbapenemases were identified consecutively.[41] Our study demonstrated that *bla*NDM-like-producing *E. cloacae* accounted for the highest proportion (60.0%), followed by *bla*KPC-like -producing *E. cloacae* (28.0%), which raises concerns regarding their dissemination patterns within clinical settings. Furthermore, five strains of *K. pneumoniae* simultaneously producing *bla*KPC-like plus *bla*NDM-like were identified. Notably, these strains exhibited resistance to all β-lactam antibiotics and ceftazidime-avibactam, necessitating heightened vigilance. Furthermore, an *bla*IMP-like-producing strain was detected within *Klebsiella oxytoca* and *Serratia marcescens* isolates in our study.

In general, CRE strains exhibit high levels of resistance to the vast majority of antibiotics. Previous research has shown that tigecycline, colistin, and aminoglycoside drugs are highly effective against these bacteria.[42] However, our findings indicate that the resistance rate of tigecycline has reached 5.8%, which may be attributed to the irrational use. Our findings indicate that *bla*KPC-like-producing strains demonstrate significant resistance to carbapenems, particularly meropenem. Polymyxin is widely recommended as a last-resort treatment option, and it can be used in combination with other medications.[43] Furthermore, several reports highlighted the potent in vitro activity of new β-lactam/β lactamases combinations (eg ceftazidime-avibactam, meropenem-vaborbactam and imipenem-relebactam).[22,44,45] Therefore, polymyxin, new β-lactam/β-lactamases, or other combination therapy could be used to treat *bla*KPC-like-producing-related infections.

Invasive bacterial infections often present significant challenges, giving rise to complex treatment and elevated mortality rates.[46] CRE infections primarily encompass sepsis, severe intracranial infections, and intraperitoneal infections.[47,48] In this study, invasive infections accounted for 19.7%, which are predominantly due to *K. pneumoniae* infections. *bla*KPC-like-producing *K. pneumoniae* constituted 50.8% of the invasive infections. Henceforth, determining the carbapenemase types of CRE strains for early intervention is urgently necessary to preserve lives.[49] In addressing this issue, We evaluated an immunochromatographic assay able to detect main carbapenemase with a turnaround time about 15 minutes, significantly shorter than other common used methods such as molecular testing and other phenotypic methods. These advantages of the immunochromatographic method allow us to envisage its implementation in rapid microbiological diagnostics, especially in cases of invasive infections. In fact, the rapid determination of the type of carbapenemase can aid the choice of effective antimicrobial therapy, including the appropriate use of recently approved new drugs (eg ceftazidime-avibactam).[50–52] Compared with PCR, immunochromatographic assay exhibits sensitivity and specificity exceeding 94.3% for KPC. NDM, IMP and OXA-48-like also demonstrate a high accuracy. Immunochromatographic assay has demonstrated valuable application potential in promptly identifying carbapenemase of CRE strains.

Notably, different types of carbapenemase may exhibit varying degrees of resistance to carbapenems.[53,54] Our observations indicate that *bla*OXA-48-like- producing strains had lower resistance to carbapenem compared with *bla*KPC-like and *bla*NDM-like-producing strains. Hence, we hypothesized that augmenting the dosage of carbapenems or combining them with other drugs such as polymyxin or ceftazidime-avibactam could be effective strategies for treating *bla*OXA-48-like-producing strains.

Our study has several limitations. Firstly, we acknowledged that our study design with a relatively limited sample size in the local area, the epidemiology of carbapenemase transmission and carriage may vary in different regions, leading to potential selection bias and a lack of generalizability to some extent. Secondly, the strains were collected based on existing phenotypes of carbapenem resistance; thus, the strains with lower carbapenemase levels or mutations may not have been identified. Thirdly, our immunochromatographic assay only detects the five most common carbapenemase types in CP-CRE strains, and other types or mutations cannot be detected. Lastly, due to limited funding, we did not conduct whole-genome sequencing analysis on all strains but only used PCR for confirmation.

CRE strains exhibit high resistance to a diverse range of antibacterial agents, with *bla*KPC-like being widely prevalent in CRE strains, particularly *bla*KPC-like-producing *K. pneumoniae*. Consequently, emphasizing the judicious use of antibacterial drugs and strengthening surveillance for CRE while enhancing prevention and control measures against nosocomial infections are necessary.

Conclusion

CRE strains demonstrated a high resistance rate to multiple antibacterial agents, with *bla*KPC-like being widely prevalent among these strains, particularly in *K. pneumoniae*. It is imperative that clinical attention be directed towards the rational use of antibacterial medications. Furthermore, continuous enhancement of CRE monitoring and hospital infection prevention and control measures are essential.

Abbreviations

CRE, carbapenem-resistant Enterobacterales; CDC, Centers for Disease Control and Prevention; CP-CRE, carbapenemase-producing CRE; non-CP-CRE, non-carbapenemase-producing CRE; KPC, *Klebsiella pneumoniae* carbapenemase; MBLs, metallo-β-lactamases; NDM, New Delhi metallo-β-lactamases; IMP, active on imipenem-type carbapenemases; CLSI, Clinical and Laboratory Standards Institute; FDA, Food and Drug Administration; USCAST, the United States Committee on Antimicrobial Susceptibility Testing; MIC, Minimum inhibitory concentration; VIM, Verona integron-encoded MBLs; OXA-48-like, Oxacillinases-48-like; Kpn, *Klebsiella pneumoniae*; Eco, *Escherichia coli*; Ecl, *Enterobacter cloacae*; CR-Kpn, carbapenem-resistant *Klebsiella pneumoniae*; CR-Eco, carbapenem-resistant *Escherichia coli*; CR-Ecl, carbapenem-resistant *Enterobacter cloacae*; CR-Eae, carbapenem-resistant *Klebsiella aerogenes*; CR-Cfr, carbapenem-resistant *Citrobacter freundii*.

Data Sharing Statement

All patient information collected by our selected strains is passed through the hospital's electronic medical recording system, and patient privacy is respected and never disclosed. All data are included in the manuscript, and some experimental materials are available upon request.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and was reviewed and approved by the Research Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University (2022121). Written informed consent was obtained from all the participants including patients' data, and in the case of participants under 18 years of age, we also informed and signed an informed consent to their parents or legal guardians prior to the enrollment of this study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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